PPILOW Workshop-
USAMV Cluj-Napoca, Romania

- Part I“Phytotherapeutic remedies used in swine low input farms, their antiparasitic efficacy”

In collaboration with:

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- *The Association of Farmers of Traditional pig breeds: Bazna and Mangalitsa*

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The antiparasitic efficacy of some medicinal and aromatic plants found in the flora of Romania against naturally occurring digestive parasites of swine

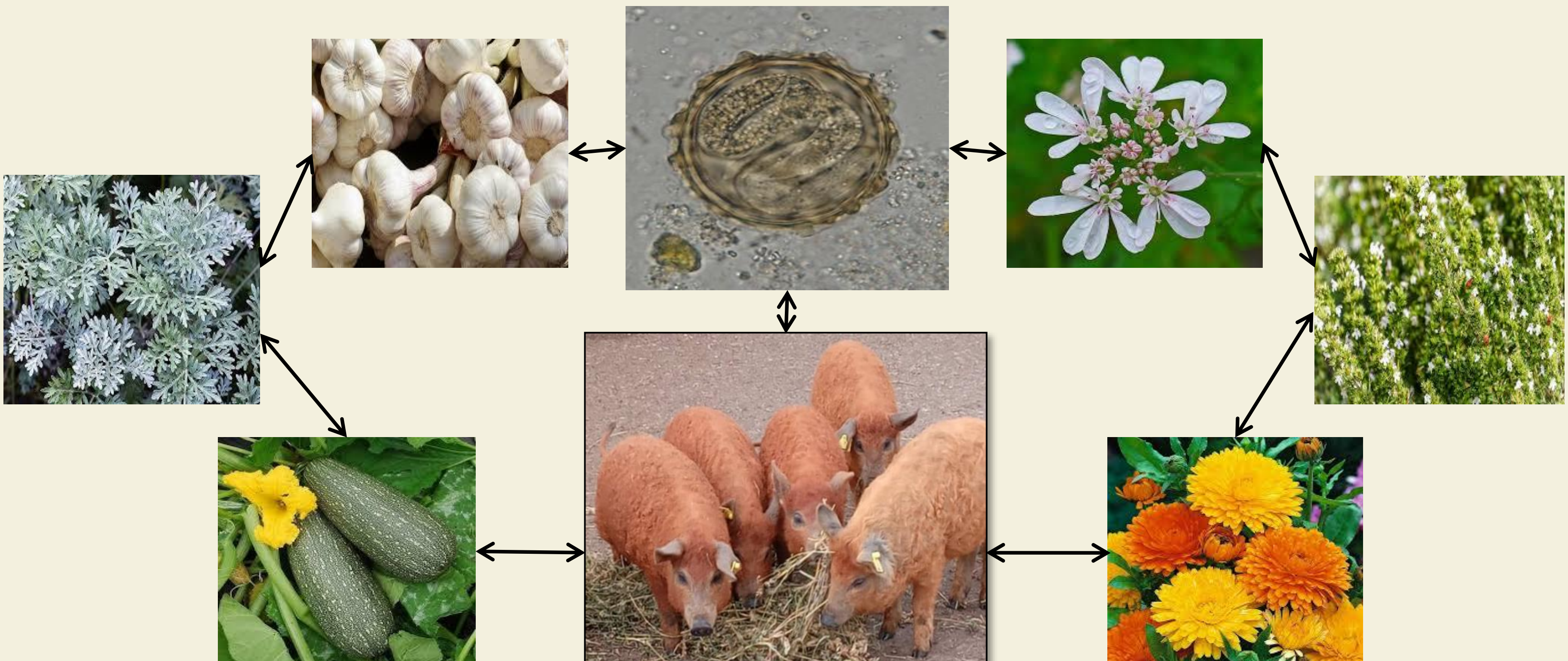
Introduction

- Parasitic diseases have a considerable effect on pig production, causing economic losses due to high morbidity and mortality.
- Due to continuously increasing drug resistence in parasites and prohibited use of antiparasitic medications in organic pig farming practices, phytotherapy could represent a valid, biologically available and cost effective alternative for parasite control.
- The use of phytotherapeutic remedies has notably increased over the past decade due to their biodegradability, decreased toxicity, environmentally friendliness, and to some extent their antiparasitic effect.

Fig. 1. Picture showing a free-range farm.

Aims

- **The primary objective of this research was to identify a plant-based formula that exhibits effectiveness in combating pig parasitoses without interfering with their welfare and health.**
- **The present study was designed to assess the antiparasitic potential of *Allium sativum, Artemisia absinthium, Cucurbita pepo, Coriandrum sativum, Calendula officinalis, and Satureja hortensis* on naturally occurring gastrointestinal parasites of swine in two free-range (low-input) farms from Transylvania**.



Materials and methods

1. Biochemical analyses of medicinal plants

- High performance liquid chromatography coupled with mass spectrometry (HPLC/MS) was used for the analysis of biologically active compounds present in the plant extracts. All the procedures were performed at the Iuliu Haţieganu University of Medicine and Pharmacy, in Cluj-Napoca.

2. Experimental design and swine husbandry

- For each farm and plant:
  - 3 control groups
    - 10 weaners, 10 fatteners and 10 sows
  - 3 experimental groups
    - 10 weaners, 10 fatteners and 10 sows
    - received *A. sativum* in a dosage of 180 mg/kg BW/day and *A. absinthium* in a dosage of 90 mg/kg BW/day for 10 consecutive days
    - received *C. sativum* in a dosage of 170 mg/kg BW/day and *C. pepo* in a dosage of 500 mg/kg BW/day for 10 consecutive days
    - received *C. officinalis* in a dosage of 140 mg/kg bw/day and *S. hortensis* in a dosage of 100 mg/kg BW/day for 10 consecutive days

Materials and methods

- 2160 faecal samples were collected from weaners, fatteners, and sows.
- Coproparasitological examination methods: flotation (Willis, McMaster), active sedimentation, modified Ziehl-Neelsen stained fecal smear, modified Blagg technique and oocysts/eggs cultures.

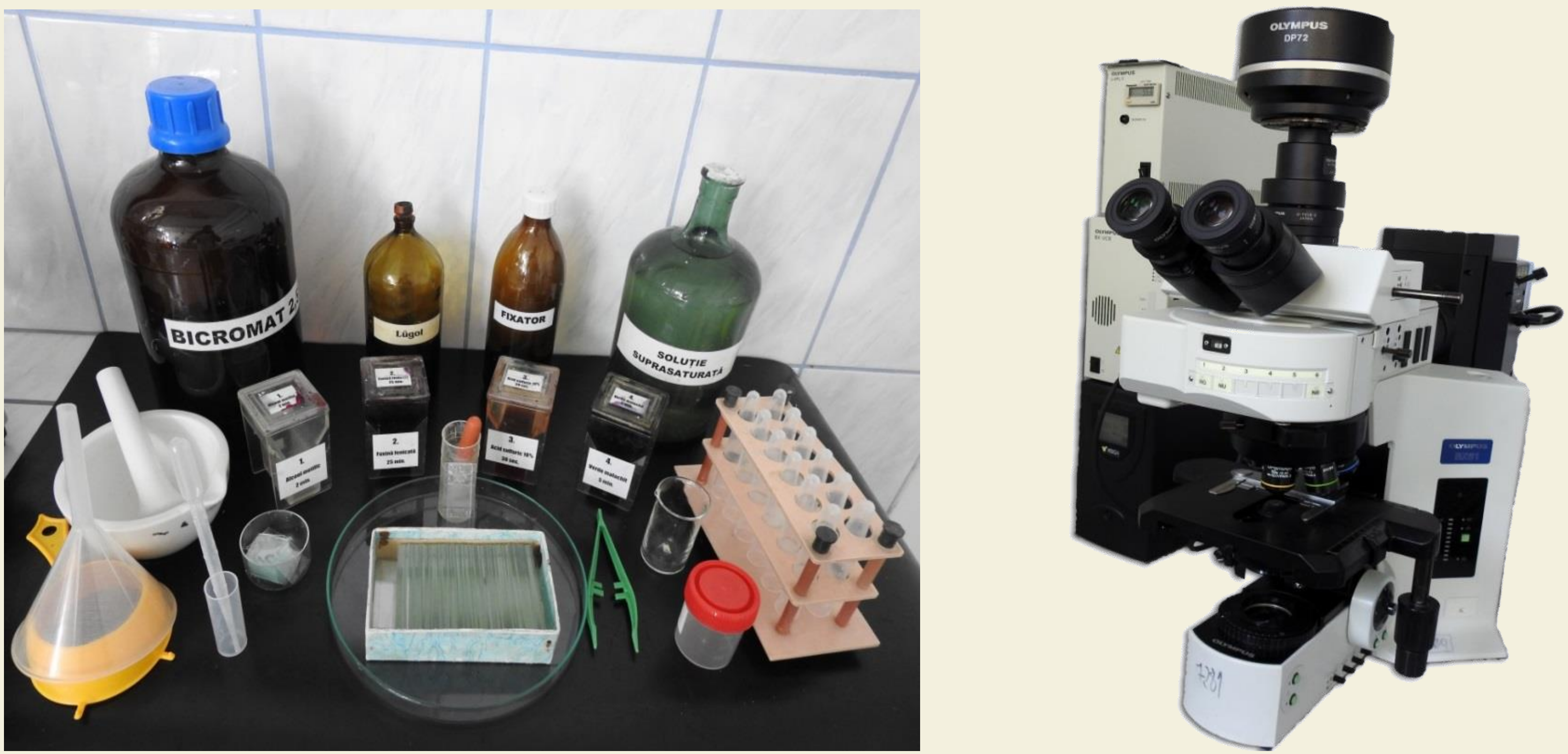


Fig. 2. Materials required for the coproparasitological methods.

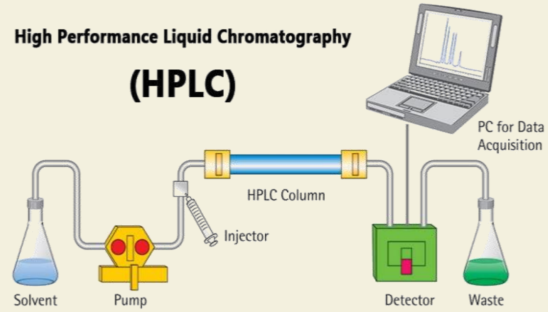
I. *In vivo* assessment of the antiparasitic effects of *Allium sativum* and *Artemisia absinthium* against gastrointestinal parasites in swine, from low-input farms, in NW of Romania

Results

Table 1. The HPLC/MS analysis of chemical compounds in alcoholic plant extracts (10%)

Bioactive compounds		Vegetal species and plant part used for extraction and HPLC-MS analysis	
		Artemisia absinthium L.	*Allium sativum* L.
		herba	bulbus
Polyphenols (µg/mL)	Chlorogenic acid	107.15	-
	Caffeic acid	-	1.221
	p-coumaric acid	0.621	-
	Ferulic acid	0.759	0.456
	Sinapic acid	-	0.228
	Vitexin	1.631	-
	Isoquercitrin	56.754	-
	Rutoside	3.826	-
	Quercitrin	1.113	-
	Quercetol	6.285	-
	Luteolin	1.159	-
	Kaempferol	3.666	-
	Apigenin	0.481	-
	Syringic acid	1.85	-
	Protocatechuic acid	1.32	-
	Vanillic acid	1.98	-

Results



Bioactive compounds		**Vegetal species and plant part used for extraction and HPLC-MS analysis**	
		***Artemisia absinthium* L.**	***Allium sativum* L.**
		herba	bulbus
Tocopherols (ng/mL)	α-tocopherol	50.0	36.1
	γ-tocopherol	23.8	-
	Δ-tocopherol	5.0	-
Sterols (μg/mL)	Ergosterol	0.344	-
	Stigmasterol	34.831	-
	B-sitosterol	140.985	-
	Campesterol	3.329	-
Methyoxylated flavones (ng/mL)	Jaceosidin	-	-
	Hispidulin	3047.92	-
	Eupatorin	976.53	-
	Casticin	15384.14	-
	Acacetin	-	-
Sesquiterpene lactones (ng/ml)	α-santonin	450.52	-
	Vulgarin	6499.39	-
Sulfoxide (μg/mL)	Aliin	-	14.726

HPLC/MS—high performance liquid chromatography coupled with mass spectrometry; “-”—Not found;

Results

The examination revealed parasitic infections with *Balantidium coli*, *Eimeria* spp., *Cryptosporidium* spp., *Ascaris suum*, *Trichuris suis*, *Oesophagostomum* spp. and *Strongyloides ransomi*.

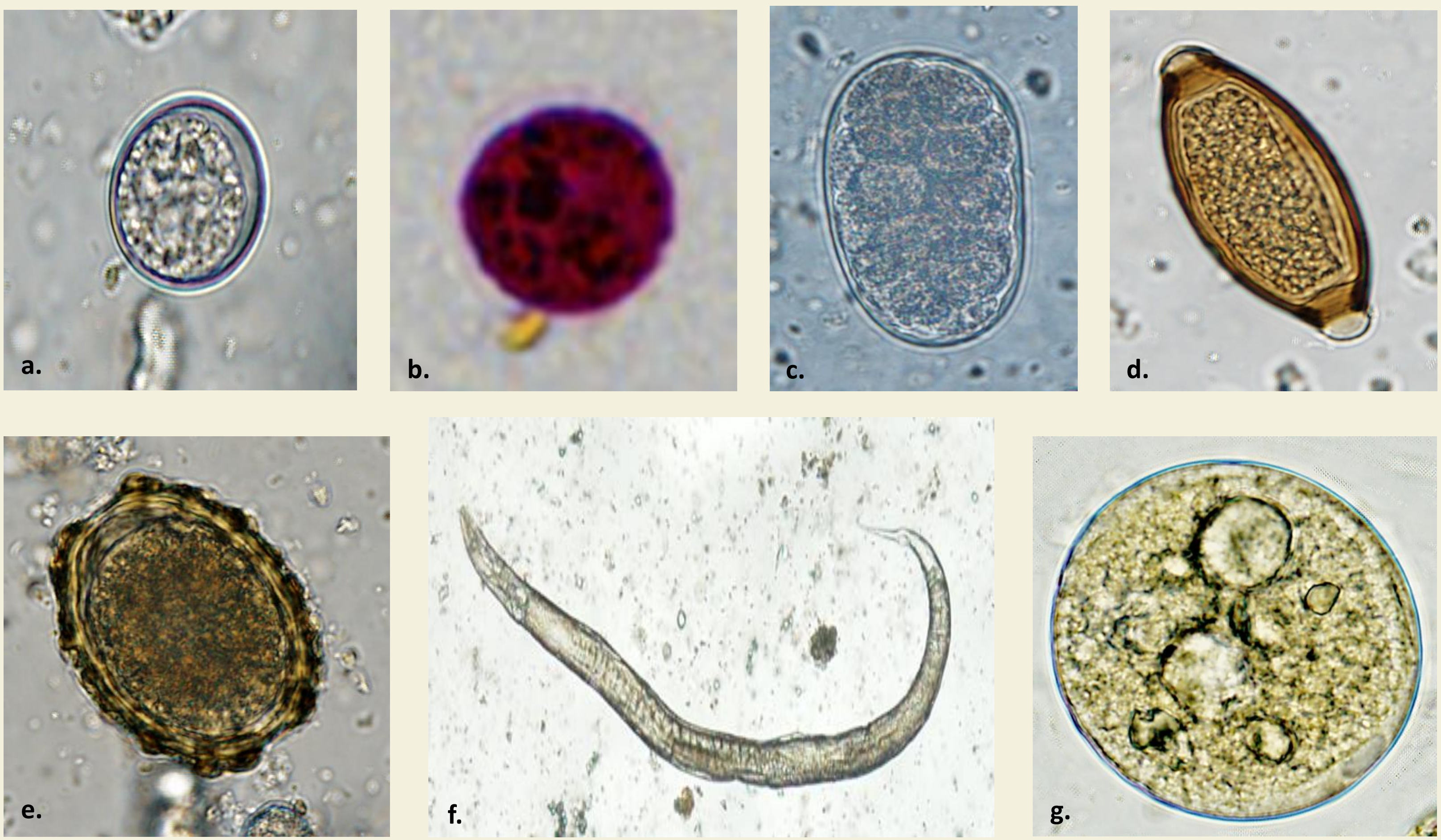


Fig. 3. Coproparasitological examination results: a- *Eimeria spp.* oocyst*, b- Cryptosporidium* spp. cyst, *c- Oesophagostomum spp.* egg, d- *T. suis egg, e- A. suum* egg*, f- S. ransomi* female and *g- B. coli.*

Results

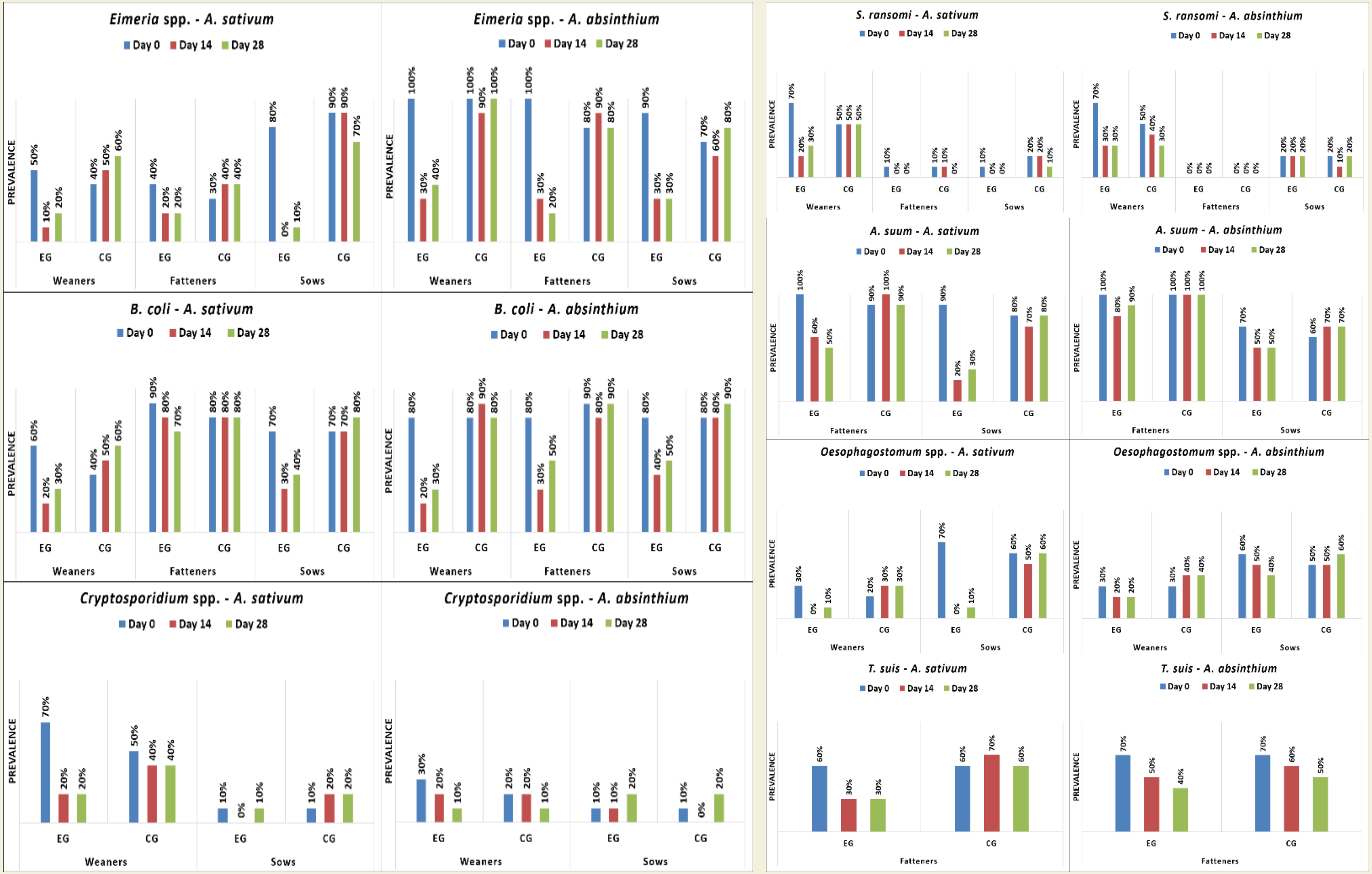


Fig. 4. Prevalence (%) of investigated protozoa and nematodes on farm 1 by age group (EG = experimental group; CG = control group).

Results

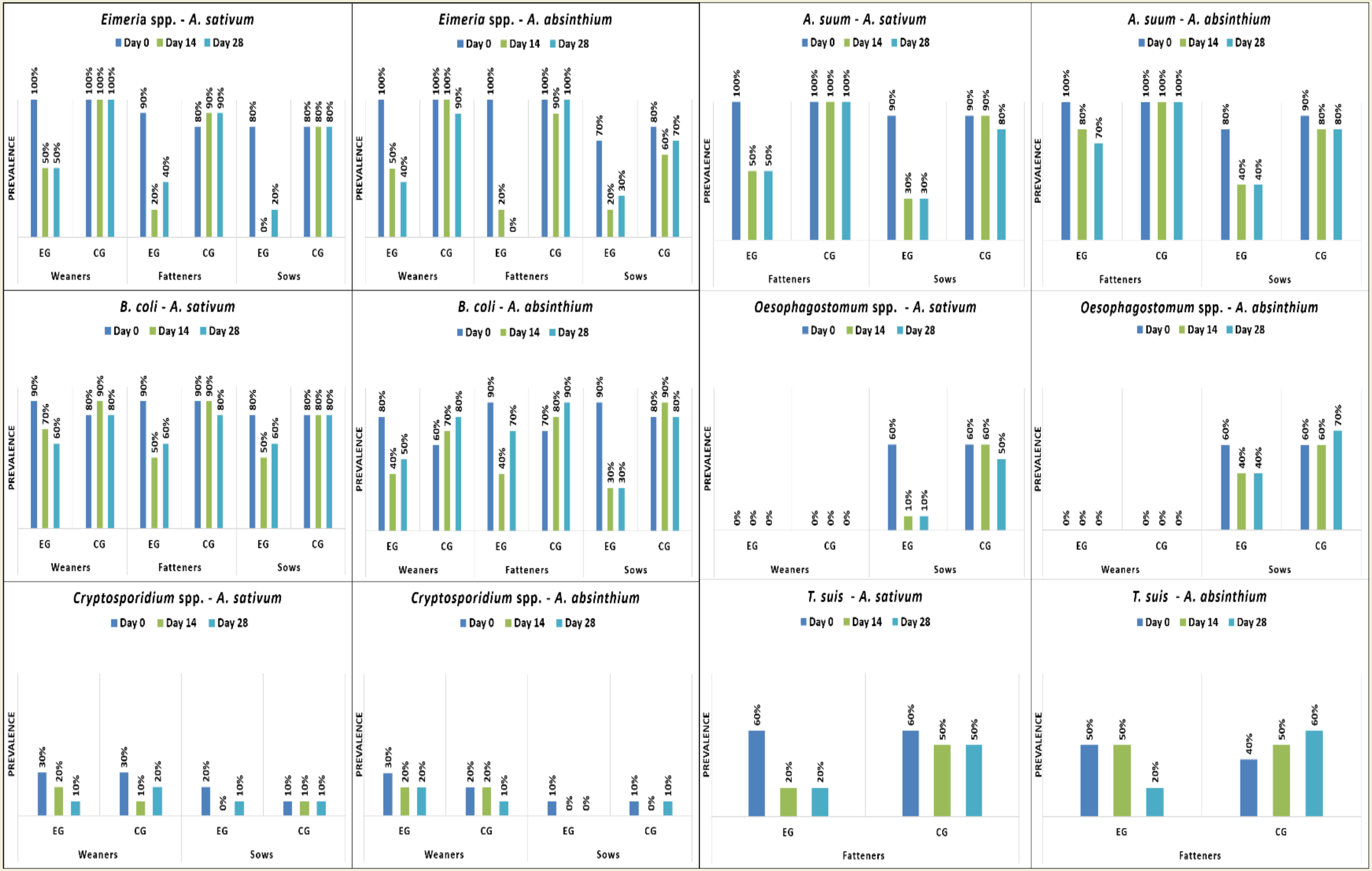


Fig. 5. Prevalence (%) of investigated protozoa and nematodes on farm 2 by age group (EG = experimental group; CG = control group).

Results

Table 2 .Percentage of faecal egg/oocyst/cyst count reduction (%) recorded on days 14, and 28 post-treatment in F1 and F2 farms (using FECR formula)

Parasite	*A. sativum* (14)						*A. sativum* (28)					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Eimeria spp.	76.7	82.1	62.1	79.6	**100**	**100**	88.1	84.6	**20.0**	84.1	78.9	83.5
B. coli	59.8	74.2	**76.1**	75.1	82.3	66.3	**47.9**	72.3	66.7	69.8	55.8	67.8
A. suum	-	-	82.3	79.8	**87.6**	72.1	-	-	84.7	86.3	68.2	**62.8**
T. suis	-	-	66.7	76.6	-	-	-	-	63.9	54.1	-	-
Oesophagostomum spp.	**100**	-	-	-	**100**	87.5	88.7	-	-	-	67.3	**45.8**
S. ransomi	64.4	-	**100**	-	**100**	-	**57.3**	-	**100**	-	**100**	-
Parasite	***A. absinthium* (14)**						***A. absinthium* (28)**					
	Weaners		**Fatteners**		**Sows**		**Weaners**		**Fatteners**		**Sows**	
	F1	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**
Eimeria spp.	74.2	84.0	71.8	**33.1**	65.8	92.4	71.5	84.9	85.1	**100**	56.3	89.8
B. coli	72.1	**88.4**	60.3	37.7	58.7	88.0	63.3	80.6	46.9	71.9	**31.6**	85.1
A. suum	-	-	71.3	64.9	44.7	**80.5**	-	-	70.4	64.3	**30.2**	78.6
T. suis	-	-	50.4	**39.5**	-	-	-	-	49.9	**79.2**	-	-
Oesophagostomum spp.	33.2	-	-	-	49.5	63.1	**25.1**	-	-	-	43.8	**66.7**
S. ransomi	36.2	-	-	-	44.4	-	**31.3**	-	-	-	69.1	-

"-"= was not diagnosed

Conclusions

- This experiment was conducted between April and July 2021, on two free-range (low-input) Transylvanian farms, involving pigs of the Bazna and Mangalitza breeds.
- Both plant powders at the previously mentioned doses for 10 consecutive days had a strong antiprotozoal and anthelmintic activity, with *A. sativum* being more effective.
- *A. sativum* and *A. absinthium* have the potential of treating gastrointestinal parasitosis in swine.
- The antiparasitic efficacy can be attributed to the presence of polyphenols, tocopherols, flavonoids, sesquiterpene lactones and sulfoxide.

II. The effects of *Coriandrum sativum* L. and *Cucurbita pepo* L. against gastrointestinal parasites in swine: An *in vivo* study

Results

Table 3. The HPLC/MS analysis of chemical compounds in alcoholic plant extracts (10%)

Chemical class	Chemical compound	Plant species and plant part used for extract preparation and the results of HPLC-MS analysis	
		***Coriandrum sativum* L.**	***Cucurbita pepo* L.**
		fruit	**seed**
Polyphenols (µg/mL)	Chlorogenic acid	4.177	-
	p-coumaric acid	0.501	-
	Ferulic acid	0.759	-
	Rutoside	<LOQ	-
	Syringic acid	0.09	-
	Vanillic acid	0.94	-
Tocopherols (ng/mL)	γ-tocopherol	-	446.0
	Δ-tocopherol	-	23.2
Sterols (µg/mL)	Ergosterol	0.584	-
	Stigmasterol	9.675	22.024
	B-sitosterol	31.548	5.355
	Campesterol	1.780	0.358

HPLC/MS—high performance liquid chromatography coupled with mass spectrometry; "-"—Not found; <LOQ—identified based on MS spectra but not determined quantitatively, below limit of quantification.

Results

The examination revealed parasitic infections with *Balantioides coli*, *Eimeria* spp., *Cryptosporidium* spp., *Ascaris suum*, *Trichuris suis*, and *Oesophagostomum* spp.

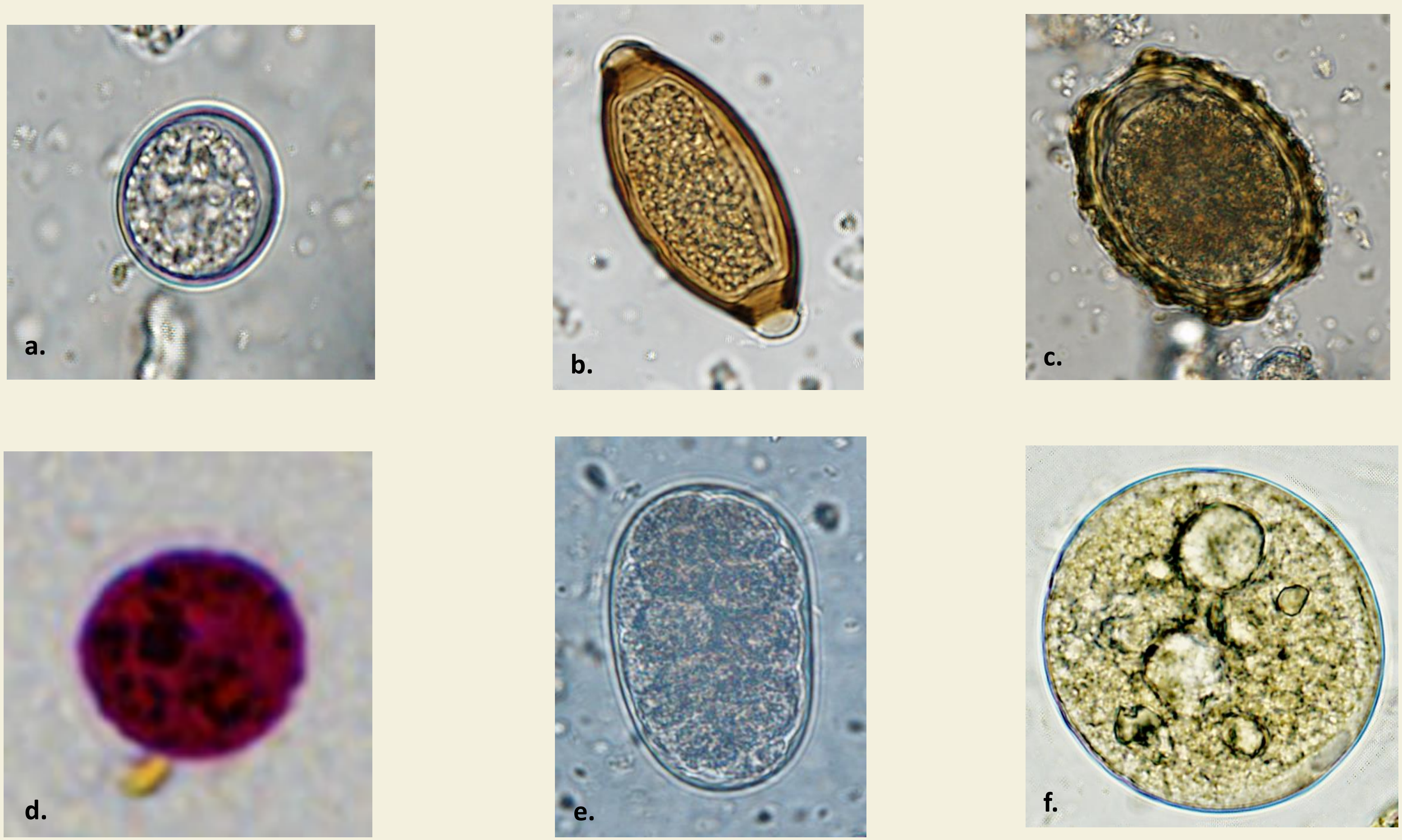


Fig. 6. Coproparasitological examination results: **a**- *Eimeria spp.* oocyst, **b**- *T. suis egg, c- A. suum* egg, **d**- *Cryptosporidium* spp. cyst, **e**- *Oesophagostomum spp.* egg, and **f**- *B. coli.*

Results

Fig. 7. Prevalence (%) of parasites in **weaners** by treatment (EG = experimental group; CG = control group).

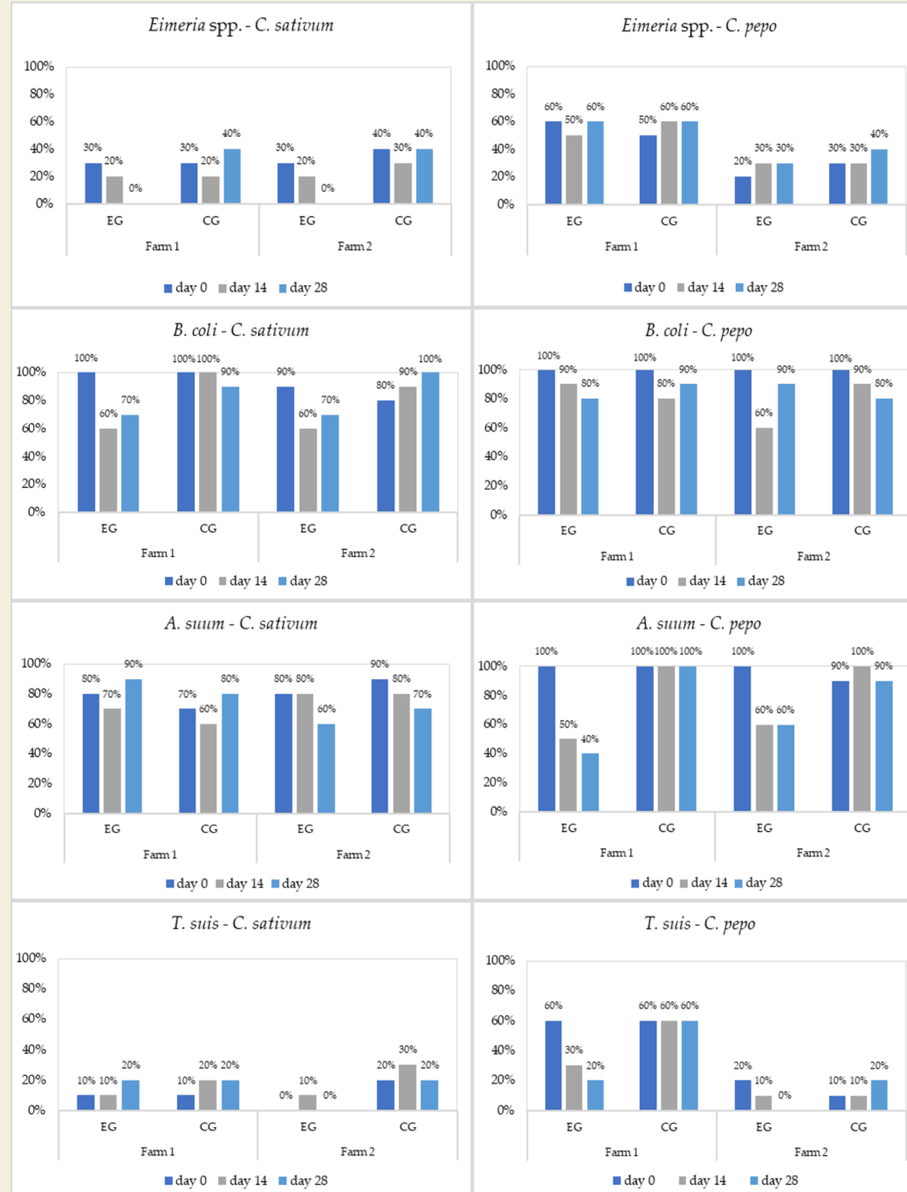


Fig. 8. Prevalence (%) of parasites in **fatteners** by treatment (EG = experimental group; CG = control group).

Results

Fig. 9. Prevalence (%) of parasites in **sows** by treatment (EG = experimental group; CG = control group).

Results

Table 4 .Percentage of faecal egg/oocyst/cyst count reduction (%) recorded on days 14, and 28 post-treatment in F1 and F2 farms (using FECR formula)

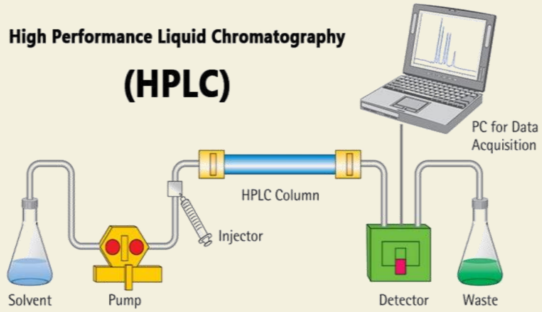
Parasite	***C. sativum* (14)**						***C. sativum* (28)**					
	Weaners		**Fatteners**		**Sows**		**Weaners**		**Fatteners**		**Sows**	
	F1	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**
Eimeria spp.	71.4	72.1	80	30.6	60	41.5	**100**	**25.4**	**100**	**100**	50	75.7
B. coli	29.6	68.9	44.4	62.4	**23.2**	74.2	**84.4**	79.5	50.4	20.1	67.4	31.2
A. suum	-	18.1	8.1	13.9	-	**0**	-	**30.3**	0	7.2	-	**0**
T. suis	-	0	0	0	-	-	-	3.3	0	0	-	-
Parasite	***C. pepo* (14)**						***C. pepo* (28)**					
	Weaners		**Fatteners**		**Sows**		**Weaners**		**Fatteners**		**Sows**	
	F1	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**
Eimeria spp.	11.6	**96.6**	13.9	33.3	45.4	**0**	24.9	94.7	35.9	**0**	61.1	0
B. coli	2.3	**59.5**	22.9	54.9	3.0	30.1	**0**	34.1	45.1	24.8	33.6	22.2
A. suum	77.4	80.9	83.5	79.7	87.1	**70.3**	79.7	**100**	84.5	95.9	85.9	88.9
T. suis	91.6	80.7	**50.1**	75.0	-	-	91.0	**100**	57.7	**100**	-	-

"-"= was not diagnosed; "0"= was identified, but had no efficacy

Conclusions

- This experiment was carried out between September and December 2021, on two low-input farms, located in the Transylvania area, involving pigs of the Bazna and Mangalitza breeds.
- Both plant powders at the previously mentioned doses for 10 consecutive days , were efficient against gastrointestinal parasites in swine. Coriander was more effective against protozoa while pumpkin showed better efficacy against helminths.
- Considering all the constraints of Romanian livestock farming, these results are a beacon of hope for better management and welfare practices in the swine farming.
- In addition, to the best of our knowledge, this is the first ethnopharmacological report on the antiparasitic effects of *C. pepo* and *C. sativum* traditionally used in Romania for treating protozoa and nematode infections in swine.

III. *Satureja hortensis* L. and *Calendula officinalis* L., two Romanian plants with *in vivo* antiparasitic potential on digestive parasites of pigs



Results

Table 5. The HPLC/MS analysis of chemical compounds in alcoholic plant extracts (10%)

Chemical class	Chemical compound	Plant species and plant part used for extract preparation and the results of HPLC-MS analysis	
		Calendula officinalis L.	*Satureja hortensis* L.
		aerial part	aerial part
Polyphenols (μg/mL)	Chlorogenic acid	220.767	<LOQ
	Caffeic acid	-	<LOQ
	p-coumaric acid	-	1.464
	Ferulic acid	-	0.557
	Isoquercitrin	38.877	6.515
	Rutoside	18.819	<LOQ
	Quercitrin	<LOQ	0.365
	Quercetol	-	0.394
	Luteolin	-	6.621
	Apigenin	-	2.442
	Syringic acid	1.51	2.28
	Protocatechuic acid	0.67	0.95
	Vanillic acid	0.44	0.65

Results

Chemical class	Chemical compound	Plant species and plant part used for extract preparation and the results of HPLC-MS analysis	
		Calendula officinalis L.	*Satureja hortensis* L.
		aerial part	**aerial part**
Tocopherols (ng/mL)	α-tocopherol	61.6	86.8
	γ-tocopherol	248.9	89.0
	Δ-tocopherol	9.3	13.2
Sterols (μg/mL)	Ergosterol	0.500	1.420
	Stigmasterol	72.888	14.215
	B-sitosterol	241.997	313.315
	Campesterol	1.635	6.140
Methoxylated flavones (ng/mL)	Jaceosidin	-	8820.76
	Hispidulin	-	2483.00
	Acacetin	-	12691.97

HPLC/MS—high performance liquid chromatography coupled with mass spectrometry; "-"—Not found; <LOQ—identified based on MS spectra but not determined quantitatively, below limit of quantification.

Results

The examination revealed parasitic infections with *Balantioides coli*, *Eimeria* spp., *Cryptosporidium* spp., *Ascaris suum*, *Trichuris suis*, and *Oesophagostomum* spp.

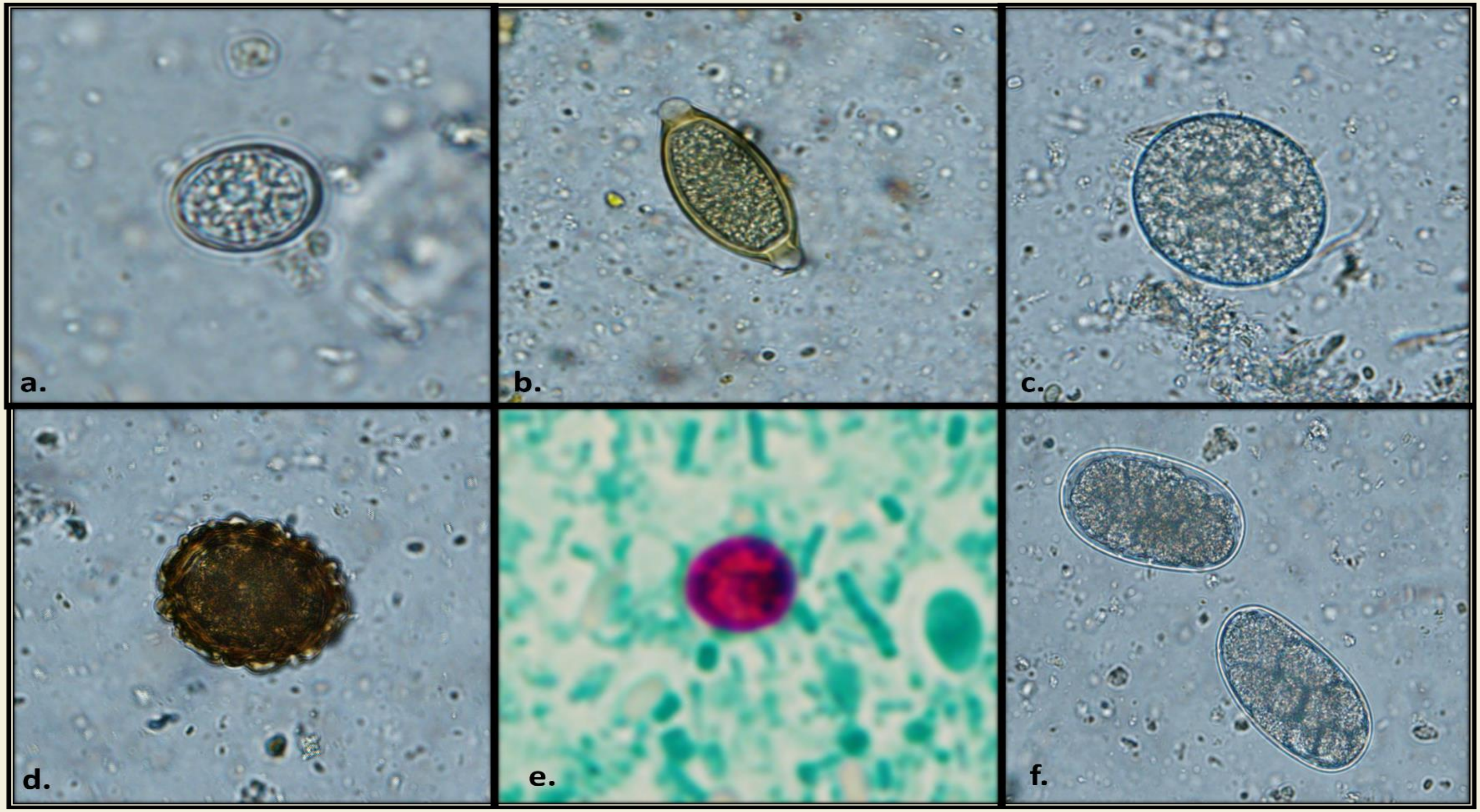


Fig. 10. Coproparasitological examination results: **a**- *Eimeria spp.* oocyst, **b**- *T. suis egg, c- B. coli* cyst, **d**- *A. suum* egg, *Cryptosporidium* spp. oocyst and **e**- *Oesophagostomum spp.* egg.

Results

Table. 6 Percentage of faecal egg/oocyst/cyst count reduction (%) recorded on days 14, and 28 post-treatment in F1 and F2 farms (using FECR formula)

Parasite	*C. officinalis* (14)						*C. officinalis* (28)					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
A. suum	-	-	15.2	10.3	-	49.9	-	-	54.2	34.9	-	79.9
T. suis	-	-	-	8.2	-	-	-	-	-	20.3	-	-
Oesophagostomum spp.	-	60.5	-	-	-	28.6	-	32.9	-	-	-	45.8
Eimeria spp.	91.8	42.5	95.5	75.9	-	74.9	72.5	57.1	88.9	30.0	-	76.5
B. coli	72.0	90.9	73.1	53.6	84.9	69.8	74.7	69.2	58.3	61.1	76.1	58.2
Parasite	***S. hortensis* (14)**						***S. hortensis* (28)**					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
A. suum	-	-	70.8	77.1	91.1	88.7	-	-	77.1	81.2	72.1	59.7
T. suis	-	-	80.5	84.0	-	-	-	-	90.3	87.1	-	-
Oesophagostomum spp.	-	-	-	-	80.2	69.2	-	-	-	-	100	83.7
Eimeria spp.	78.2	68.7	76.3	89.7	25.1	70.3	66.8	80.3	46.8	83.8	80.9	94.1
B. coli	80.1	88.4	63.5	74.7	70.2	70.5	83.6	86.5	72.2	71.2	70.7	74.6

“-“= was not diagnosed;

Conclusions

- The present experiment was conducted between April and June 2022, on two low-input (free-range) farms, located in the Transylvania area, involving pigs of the Bazna and Mangalitza breeds.
- Both plant powders at the previously mentioned doses for 10 consecutive days, showed promising *in vivo* antiparasitic activity.
- *C. officinalis* had a strong antiprotozoal activity and mildly antihelmintic effects while *S. hortensis* was very effective against both helminths and protozoa infections.
- The antiparasitic efficacy can be attributed to the presence of polyphenols, sterols, tocopherols and flavonoids.
- The current study is the first report about the antiparasitic effects of *C. officinalis* and *S. hortensis* against digestive parasites of pigs, from Romania.

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Thank you for your attention!